

U.S. Application No. 09/238,741
Supplemental Amendment dated June 14, 2004
In Reply to the Office Action of December 22, 2003
Attorney ref. no. 037003-0277113

I. AMENDMENT

IN THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

61. (Currently amended) A method for producing an antibody heterodimer comprising an anti-CD20 antibody and an anti-CD23 antibody, comprising:

(i) obtaining or constructing a DNA molecule that encodes an antibody molecule heavy chain that has binding specificity to a CD20 or CD23 antigen when said heavy chain is paired with a corresponding light chain, and introducing at least one cysteine codon into said antibody molecule heavy chain via recombinant DNA mutagenesis, ~~wherein the location of said cysteine does not interfere with the antigen binding properties of said antibody dimer;~~

(ii) expressing said DNA molecule in a suitable host cell, or expression system, together with a DNA molecule that encodes an antibody molecule light chain having the same specificity as the heavy chain, to produce a first anti-CD20 or anti-CD23 antibody molecule containing said introduced cysteine residue;

(iii) purifying said first antibody molecule from said host cell or expression system;

(iv) contacting said purified antibody molecule with an amount of a suitable reducing agent sufficient to partially reduce the intra or inter molecular disulfide bonds of said antibody molecule and thereby enhance dimerization of said first antibody molecule with a second antibody molecule; and

(v) contacting said purified first antibody molecule with a second antibody molecule that has a binding specificity to CD20 when said first antibody is an anti-CD23 antibody, or to CD23 when said first antibody is an anti-CD20 antibody, and allowing sufficient time for dimerization to proceed to thereby to produce a tetravalent antibody heterodimer comprising both an anti-CD20 antibody and an anti-CD23 antibody;

wherein each antibody molecule retains its binding specificity following dimerization, the anti-CD20 antibody of the heterodimer has binding specificity to CD20 and the anti-CD23 antibody of the heterodimer has binding specificity to CD23.

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62. (Previously presented) A method according to claim 61, wherein said first and second antibodies are both IgG antibodies.

63. (Previously presented) A method according to claim 62, wherein said first and second antibodies are both IgG-1 antibodies.

64. (Previously presented) A method according to claim 61, wherein the anti-CD20 antibody has binding specificity to a human CD20 antigen and the anti-CD23 antibody has binding specificity to a human CD23 antigen.

65. (Previously presented) A method according to claim 64, wherein the anti-human CD20 antibody is a chimeric antibody consisting of light and heavy chains of a human IgG antibody in which the variable regions are replaced with the light and heavy chain variable regions of murine C2B8 antibody; and

the anti-human CD23 antibody is a chimeric antibody consisting of light and heavy chains of a human IgG antibody in which the variable regions are replaced with the light and heavy chain variable regions of monkey 5E8 antibody.

66. (Previously presented) A method according to claim 65, wherein said first and second antibodies are both IgG-1 antibodies.

67. (Previously presented) A method according to claim 61, wherein the location of said cysteine molecule is such that it prevents or inhibits formation of an intramolecular disulfide bridge between sister heavy chains on the same antibody molecule.

68. (Previously presented) A method according to claim 61, wherein step (iv) further comprises terminating the reducing reaction by the addition of cysteine blocking reagent

69. (Previously presented) A method according to claim 61, wherein step (v) comprises cross-linking the reduced antibody molecules using a BIS-maleimido cross-linker.

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70. (Previously presented) A method according to claim 61, wherein the second antibody contains a thiol reactive group other than a cysteine group introduced therein.

71. (Previously presented) The method of Claim 70, wherein the thiol reactive group is selected from the group consisting of a maleimido group, a dithiopyridal group, and a reactive thiol.

72. (Previously presented) A method according to any one of claim 70, wherein step (iv) further comprises terminating the reducing reaction by the addition of thiol blocking reagent

73. (Previously presented) A tetravalent antibody heterodimer produced by the method of claim 61.

74. (Previously presented) An antibody heterodimer according to claim 73, wherein said first and second antibodies are both IgG antibodies.

75. (Previously presented) An antibody heterodimer according to claim 74, wherein said first and second antibodies are both IgG-1 antibodies.

76. (Previously presented) An antibody heterodimer produced by the method of claim 65.

77. (Previously presented) An antibody heterodimer according to claim 76, wherein said first and second antibodies are both IgG-1 antibodies.

78. (Previously presented) A tetravalent antibody heterodimer comprising a first antibody that has binding specificity to a CD20 antigen and a second antibody that has binding specificity to a CD23 antigen.

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79. (Previously presented) The antibody heterodimer of claim 78, wherein said first or said second antibody comprises an antibody heavy chain polypeptide that is mutated by introduction of a cysteine residue.

80. (Previously presented) The antibody heterodimer of claim 78, wherein said first and second antibodies are both IgG antibodies.

81. (Previously presented) The antibody heterodimer of claim 80, wherein said first and second antibodies are both IgG-1 antibodies.

82. (Previously presented) The antibody heterodimer of claim 78, wherein said first and second antibodies are monoclonal antibodies.

83. (Previously presented) The antibody heterodimer of claim 78, wherein the anti-CD20 antibody has binding specificity to a human CD20 antigen and the anti-CD23 antibody has binding specificity to a human CD23 antigen.

84. (Previously presented) The antibody heterodimer of claim 83, wherein the anti-human CD20 antibody is a chimeric antibody consisting of light and heavy chains of a human IgG antibody in which the variable regions are replaced with the light and heavy chain variable regions of murine C2B8 antibody; and

the anti-human CD23 antibody is a chimeric antibody consisting of light and heavy chains of a human IgG antibody in which the variable regions are replaced with the light and heavy chain variable regions of monkey 5E8 antibody.

85. (Previously presented) The antibody heterodimer of claim 84, wherein said first and second antibodies are both IgG-1 antibodies.

86. (Previously presented) The antibody heterodimer of claim 84, wherein said first and second antibodies are monoclonal antibodies.

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87. (Previously presented) The antibody heterodimer of claim 84, which activates components of the complement system.

88. (Previously presented) The antibody heterodimer of claim 84, which promotes killing of cells by the complement cascade.

89. (Previously presented) The antibody heterodimer of claim 84, which binds to Fcγ receptors on cytotoxic effector cells.

90. (Previously presented) The antibody heterodimer of claim 84, which binds to Fcγ receptors on host immune cells.

91. (Previously presented) The antibody heterodimer of claim 84, which induces B lymphoma cells to initiate apoptosis.

92. (Previously presented) The antibody heterodimer of claim 84, which induces initiation of apoptosis of leukemic cells of a patient with chronic lymphocytic leukemia.